Mammalian Cas9 Genome Editing Vectors

ATUM's Cas9 vectors express the Cas9 nuclease and the RNA sequences that guide the nuclease to its genomic target. Cas9 expression is driven by a choice of promoters and can be monitored by linked expression of green or red fluorescent proteins. Nickase and ds-nuclease versions of Cas9 are both available.

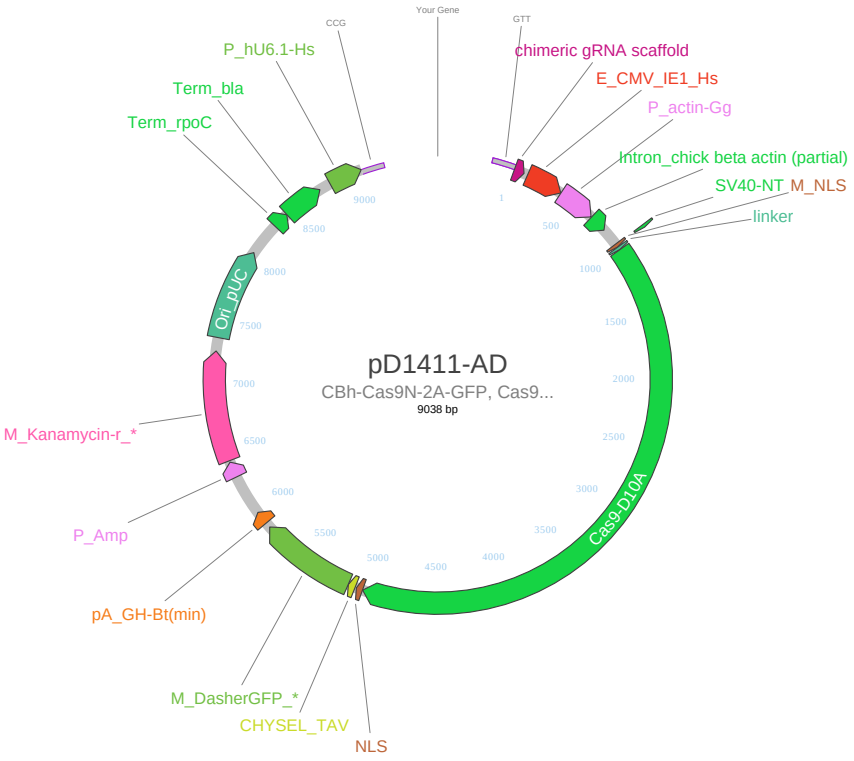
Nickase Ninjas

In our most convenient format (available only for gene synthesis orders), we provide the increased specificity of two independent guide RNAs plus the Cas9-nickase in a single vector.

SS Nickase

One guide RNA targets Cas9 to cleave one strand of the genome at the host target site. Two gRNAs targeted to nearby sites result in genomic double stranded breaks with greater specificity than can be achieved with a single guide directing Cas9 ds nuclease. Off-site effects can be further minimized by using the nickase design feature on ATUM's gRNA design tool.

Plasmid Map



Name	Qty	Storage
pD1411-AD	10Rx	−20℃

ATUM
37950 Central Court
Newark, CA 94560

1 877 362 8646 (Toll free)
info@atum.bio
www.atum.bio

gRNA Amplification Protocol

Oligo pairs designed using ATUM's gRNA Design Tool have SapI sites added on to enable cloning into gRNA Electra vectors. Just perform a regular PCR reaction with the oligo pair as primers, no template and short cycle time.

1. 96°C, 2'

2. 96°C, 15"

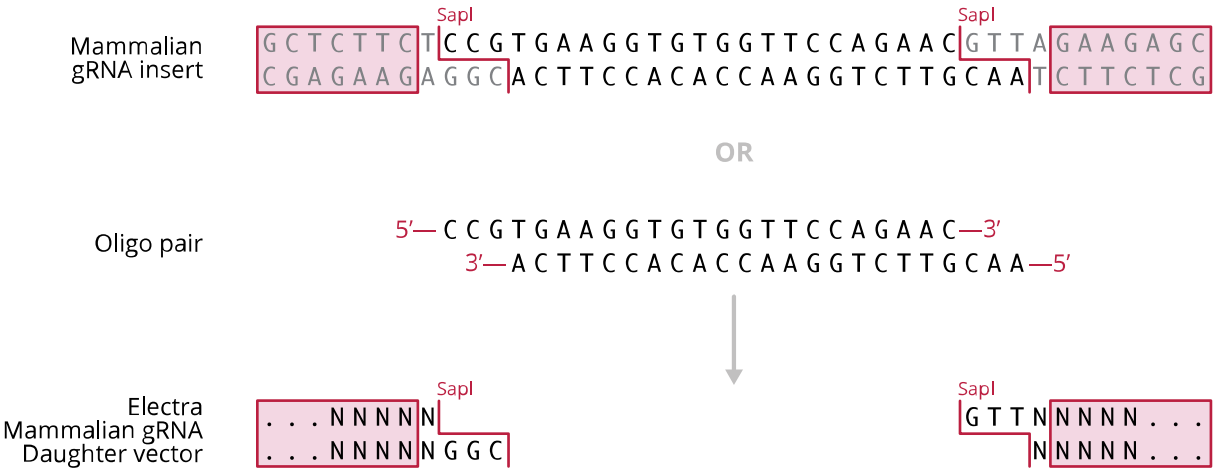
3. 58°C, 15"

4. 72°C, 1"

5. Goto step 2, 10 more times.

One microliter of this PCR reaction can be used directly in the Electra reaction without purification.

Electra Mammalian gRNA DAUGHTER Vectors



Electra gRNA DAUGHTER vectors are supplied as linearized DNA, with overhangs compatible with a CCG (including the G at the transcription start site) at the 5' end and AAC at the 3' end. Guide oligos can be incorporated by annealing a pair of oligos with the correct overhangs, or by PCR amplification followed by an Electra reaction*.

*Your gRNA sequence must not contain any internal SapI recognition sites, since the Electra cloning process utilizes the typeIIs enzyme SapI.

Cas9 Transfection Protocol

1. 24 hours before transfection seed cells (in antibiotic free media) to be 70-90% Confluent at time of transfection. (6 well plate: 0.25-1.0x10^6, 24 well plate: 0.5-2.0x10^5, 96 well plate: 1-4x10^4)

2. *Dilute Lipofectamine in DMEM or Opti-MEM (LifeTech)

3. *Dilute DNA (endotoxin free is optimal) in DMEM or Opti-MEM (LifeTech)

4. Add diluted DNA mixture to diluted Lipofectamine mixture.

5. Incubate for 10 minutes.

6. Add DNA-Lipid complex to cells

7. Visualize cells using microscope and analyze.

*At ATUM we typically use a 1:1 ratio of DNA:Lipofectamine

ATUM
37950 Central Court
Newark, CA 94560

1 877 362 8646 (Toll free)
info@atum.bio
www.atum.bio

Electra Cloning System

Electra is a simple one-tube universal cloning process that can be performed in a 5 minute bench-top reaction with the fidelity of a restriction-based cloning system. A gene from one MOTHER vector is compatible with all DAUGHTER vectors, allowing rapid testing of many different sequence contexts simultaneously.

Reagents

The Electra Reagents kit contains all necessary components to facilitate cloning a gene from a MOTHER into a DAUGHTER vector. The Electra reaction can also be used to clone a PCR product into either a MOTHER or a DAUGHTER vector.

Electra Buffer Mix is supplied at 10X final concentration (use 2 µl in a 20 µl reaction)

Electra Enzyme Mix is supplied at 20X final concentration (use 1 µl in a 20 µl reaction)

Cloning Protocol

Component	Volume (µl)
MOTHER DNA / Positive control / gRNA (20 ng)	1
DAUGHTER Vector (20 ng)	1
Electra Buffer (10x)	2
Electra Enzyme (20x)	1
Water	15
Total	**20**

1. Combine components and incubate at 25-37°C for 5-20 minutes.

2. Transform 1-2 µl into chemically competent E. coli. (DH10B cells recommended)

3. Recover cells for 45 minutes and then plate on appropriate antibiotic for the DAUGHTER.

3a. Optionally include streptomycin at 100 µg/ml (for selection against pMOTHER with rpsL); or plate on YEG with antibiotic plus p-chloro phenylalanine at 10mM (for selection against pMOTHER with pheS).

Positive Control

A positive control MOTHER vector carries a gene in which Ptet drives expression of green fluorescent protein (DasherGFP). A successful Electra reaction will produce green fluorescent colonies from the DAUGHTER vector.

ATUM
37950 Central Court
Newark, CA 94560

1 877 362 8646 (Toll free)
info@atum.bio
www.atum.bio

Feature list descriptions

Cas9-D10A	A D10A mutant of Cas9 (Cas9n) nicks single strands and combined with a pair of offset guide RNAs complementary to opposite strands of target genomic loci helps reduce off-target activity seen with wild type Cas9. Nicking of both DNA strands by a pair of Cas9 nickases leads to site-specific double stand breaks (DSBs) and NHEJ (non-homologous end joining). (www.sciencedirect.com/science/article/pii/S0092867413010155)
chimeric gRNA scaffold	The chimeric guide RNA (gRNA) scaffold consists of a 20-nt target specific complementary region, a 42-nt Cas9-binding RNA structure and a 40-nt transcription terminator derived from S. pyogenes that directs Cas9 nuclease to the target site for genome modification. (www.sciencedirect.com/science/article/pii/S0092867413010155)
CHYSEL_TAV	The TAV CHYSEL (cis-acting hydrolase element) or 2A peptide was found in the insect Thosea asigna virus (TaV). Multiple proteins can be efficiently produced from one coded peptide by a mechanism that relies on the self-cleaving 2A peptide sequence which allows for translational 'skipping'. The 2A peptide mediates the co-translational cleavage of a polyprotein. After cleavage, the short 2A peptide remains fused to the C-terminus of the 'upstream' protein, while a proline is added to the N-terminus of the 'downstream' protein. (www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0018556)
DasherGFP	IP-Free© green fluorescent reporter protein that is used as a selectable marker for expression monitoring of your protein. Ex/Em: 505/525 nm.
E_CMV_IE1_Hs	The cytomegalovirus (CMV) enhancer element plays a critical role in overcoming inefficient transcriptional activities of promoters, thereby enhancing transcription. The hCMV IE1 enhancer/promoter is one of the strongest enhancer/promoters known and is active in a wide range of cell types. (www.link.springer.com/article/10.1007%2Fs11248-008-9235-y)
Intron_chick beta actin (partial)	A truncated version of the chick beta actin intron which shows poor expression. Contains an enhancer element, which is highly conserved among vertebrates. Introns usually contain splicing signals.
Kanamycin-r	An effective bacteriocidal agent that inhibits ribosomal translocation thereby causing miscoding. The gene coding for kanamycin resistance is Neomycin phosphotransferase II (NPT II/Neo). *E.coli* transformed with plasmid containing the kanamycin resistance gene can grow on media containing 25 µg/ml kanamycin. Kanamycin is a white to off-white powder that is soluble in water (50mg/ml). (www.en.wikipedia.org/wiki/Kanamycin)
NLS	A nuclear localization signal (NLS) is an amino acid sequence that 'tags' a protein for import into the cell nucleus by nuclear transport. Typically, this signal consists of one or more short sequences of positively charged lysines or arginines exposed on the protein surface. Chelsky et al. proposed the consensus sequence K-K/R-X-K/R for monopartite NLSs. A protein translated with a NLS will bind strongly to importin, and together the complex will move through the nuclear pore. Once in the nucleus, Ran-GTP binds to the importin-protein complex, causing importin to lose affinity for the protein, thereby releasing the protein. (www.jbc.org/content/282/8/5101.full)
Ori_pUC	The origin of replication is a sequence in a genome at which replication is initiated. The pUC ori is a mutated form of origin derived from *E. coli* plasmid pBR322 which allows production of greater than 500 copies of plasmid per cell. (www.en.wikipedia.org/wiki/Origin_of_replication)
P_actin-Gg	The beta-actin promoter is a strong promoter and shows higher promoter activity than the simian virus 40 (SV40) early promoter. (www.jbc.org/content/264/16/9539.full.pdf+html)
P_hU6.1-Hs	A type 3 core promoter for RNA expression that was originally identified in mammalian U6 snRNA genes, which encode the U6 snRNA component of the spliceosome. A hallmark of type 3 is the presence of a TATA box. (www.genesdev.cshlp.org/content/16/20/2593.long)
pA_GH-Bt(min)	The bovine growth hormone polyadenylation (bgh-PolyA) signal is a specialized termination sequence for protein expression in eukaryotic cells. (www.ncbi.nlm.nih.gov/pubmed/17407167)

Licenses

pD1300, 1400, 2100, 2500, 2600 & ATUM Proprietary Mammalian Expression Vectors - RESEARCH USE ONLY

Any product containing pD1300, pD1400, pD2100, pD2500, pD2600, pD3500, or pD3600-series Vectors (the "Licensed Vectors") (including Electra vectors, vector configurations for expression of multiple genes and other customized configurations of the Licensed Vectors, and ProteinPaintbox genes or CUSTOMER genes cloned into the Licensed Vectors) is subject to a limited, non-transferable license pursuant to which CUSTOMER acknowledges and agrees that the Licensed Vector may be used for internal research purposes only and may not be used for commercial purposes. For clarity, use for commercial purposes includes any use in manufacturing a product or service that is provided to a third party for consideration. In addition, CUSTOMER acknowledges and agrees that CUSTOMER and any Authorized Transferee (as defined in Section 11) may not (a) modify the Licensed Vectors in any way, including but not limited to replacing any protein-encoding sequence with any other protein-encoding sequence; (b) reverse-engineer, deconstruct, or disassemble the Licensed Vectors; (c) create any variant or derivative vector of the Licensed Vectors; or (d) transfer, disclose, or otherwise provide access to the Licensed Vectors (including sequences of same) to any third party other than an Authorized Transferee, and provided that any transfer to an Authorized Transferee must comply with the Product Transfer terms of Section 11.